BIOACTIVITY OF SURFACE MODIFIED POROUS TITANIUM

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Certificate

This is to certify that the thesis entitled "**BIOACTIVITY OF SURFACE MODIFIED POROUS TITANIUM**" by **Pranjali Nanda (110BM0011),** in partial fulfillment of the requirements for the award of the degree of Bachelor of Technology in Biomedical Engineering during session 2010-2014 in the Department of Biotechnology and Medical Engineering, National Institute of Technology Rourkela, is an authentic work carried out by her under my supervision and guidance. To the best of my knowledge, the matter embodied in the thesis has not been submitted to any other University/Institute for the award of any degree or diploma.

Place: NIT Rourkela **Dr. A. Thirugnanam**

Date: 12th May 2014 **Assistant Professor**

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Place: NIT Rourkela **Pranjali Nanda**

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Abstract

Solid metals have widely been used for implant fabrication in the replacement of human hard tissues. For the implant-bone interface bonding, porosity plays an important role for better osseointegration. With tissue ingrowth into the porous structure of the implant this bonding has subsequently been enhanced. Pore interconnectivity facilitates extensive body fluid transport through the metallic implants. In this study, porous titanium (Ti) samples with interconnected 3D pores were taken for various surface chemical treatments that will enhance the surface chemistry and topology. Ti-samples were subjected to alkali, dual acid, citric acid and fluoride treatment to study the role of surface modified titanium in *in-vitro* bioactivity and protein adsorption. Porosity measurement being a vital parameter was also calculated with the surface porosity (optical microscope) being 41.36% and the bulk porosity (Archimedes' Principle) as 50.347%. X-ray powder diffraction (XRD) analysis shows the diffraction pattern of the samples corresponding to titanium along with those that correspond to respective surface treatments. Scanning electron microscopy (SEM) was done on samples before and after bioactivity study in simulated body fluid (SBF). The fluoride treated sample showed enhanced bioactivity as compared to other surface treatments for the same time period immersed in SBF. Protein adsorption study was also carried out with Bradford's reagent using Bovine Serum Albumin (BSA). The sample with alkali treatment showed maximum adsorption due to hydrolyzed surface enhancing protein adsorption.

Keywords: Titanium, porosity, SBF, bioactivity, protein adsorption

CHAPTER 1

INTRODUCTION

Titanium is a chemical element having the symbol Ti and atomic number 22. Titanium is a transition metal which is lustrous and silvery in color having low density and high strength. It is highly resistant to corrosive environments. Titanium was discovered in Cornwall, Great Britain by a person named William Gregor in 1971. It usually occurs with a number of mineral deposits mainly rutile and ilmenite which are widely found in living bodies, rocks, water bodies and soil. The metal is principally obtained from its ore by Kroll's process.

Kroll's process is principally a pyro-metallurgical industrial process used to manufacture metallic titanium. Kroll's process has replaced Hunter's process to commercially produce titanium [1] . Refined rutile is reduced with petroleum-derived coke at 1000^{o}C in a fluidized bed reactor. The mixture is then treated with chlorine gas, affording $TiCl_4$ and other volatile chlorides, which subsequently are separated by continuous fractional distillation. The $TiCl_4$ is reduced by liquid magnesium or sodium (15-20% excess) at 800-850 °C in a stainless steel retort for complete reduction [2]. The resulting porous metallic titanium sponge is further purified by heated vacuum distillation or leaching. The sponge is crushed and pressed before it is melted in a consumable electrode vacuum arc furnace. The melted substance is allowed to solidify in the presence of vacuum followed by further remelting to remove inclusions and bring uniformity. The melting steps make the process costly making titanium six times as costly as stainless steel.

Stress shielding is the reduction in density of bone due to removal of normal stress from the bone by an implant due to the difference in their modulus of elasticity. This is because if the loading on the bone decreases then as a result of this the bone mass decreases and becomes weaker due to the lack of enough stimuli needed for maintaining the bone mass. A bone in its natural state is meant to carry the external stresses all by itself. Initially, where only one structure was supposed to carry the load, i.e. the bone, now it is carried by two, the implant as well as the bone. Since

the bone now is subjected to some amount of reduced stresses, it leads to stress shielding. Stress shielding is thus a mechanical phenomenon. In accordance with Wolff's Law, the reduction of the stresses relative to the natural condition would lead to bone adapting itself to the situation by a reduction in its mass, either by turning more porous (internal remodeling) or turning thinner (external remodeling). This bone remodeling continues over the years and after a deduction in about 50%, it becomes quite prone to falls and other accidents leading to injury and implant loosening. The combination of both bone resorption around the prosthetic stems and stress shielding lead us to believe that there is a biomechanical phenomenon involved in it [3].

Mismatch (Stress Shielding) of elastic modulus between bone and implant result in loosening of bone-implant interface posing as a critical hindrance in clinical application [4]. Porous titanium has gained the attention as method to reduce stress shielding as well achieve a stable long term fixation with desired bone ingrowth. Solid state foaming, dipping method with polymerfoam, loose pack sintering are few of the methods used to fabricate porous titanium implants [5]. Porous titanium implants give desired pore structure and impressive mechanical strength.

Ti and Ti alloys possess low modulus in the range 110 to 55 GPa. Commercially pure Ti and Ti–6Al–4V are most commonly used for implant applications. High corrosion resistance and excellent biocompatibility of titanium increases its suitability for biomedical applications. The mechanical strength of the Ti and its alloys is near to 316L SS, and its density is 55% less than steel. The applications include joint replacement parts for hip, knee, elbow, spine, shoulder, dental implants, etc. Although titanium and its alloys, mainly Ti6Al4V have an excellent corrosion resistance and biocompatibility, a long term use result in release of Al and V ions. Both vanadium and aluminum ions released from the Ti6Al4V alloy are cause health issues like

Alzheimer's disease, etc. Toxicity of vanadium is also present in the elemental state and oxides V_2O_5, present at the surface. The bioinertness of Ti also restricts the use [6].

Beside commercially pure titanium (c.p-Ti) and Ti6Al4V, β-titanium alloys such as Ti-Ta alloys; Ti-Mo alloys; Ti-Nb and Ti-Ni shape memory alloys are very impressive as bioimplants [6, 7]. These are preferred due to high corrosion resistance and biocompatibility. Ti-Ta alloys have lower modulus and a good package of high strength and low modulus. They have potential to become new entries for biomedical applications. Addition of Zr to Ti alloy lowers the Young's modulus and other mechanical properties thus making it more suitable for biomedical applications [6].

Nickel-titanium (Ni-Ti) shape memory alloys have been proved as a very important bone implant due to its excellent mechanical properties, good corrosion resistance, high biocompatibility, special pseudoelasticity and shape including its volume memory effect. Its porous structure allows the ingrowth of new- bone tissues along proper nutrient supply. By obtaining different porosity through processing parameters, the elastic modulus of the final porous Ni–Ti is matched with the human bone [4].

Recent advancement in this arena has brought Ti-hydroxyapatite (HA) metal matrix composite into the scenario. The superior biocompatibility and bioactivity of HA is combined with the inert and higher mechanical properties of titanium to result in a composite that has better biocompatibility, bioactivity and impressive mechanical properties [8, 9]. This composite is non-toxic and highly bioactive. The porous morphology facilitates the bone in-growth and supports osseointegration. The *in vitro* results in simulated body fluid (SBF) show dense hydroxyapatite particles formation on the implant [8, 10]. The tensile strength, Young's modulus decreases with

increase in volume fraction of HA. This composite is also thermodynamically and electrochemically stable in physiological environment [11].

Bioactivity of a substance refers to the effect it has on living tissues. For any implant using metal that usually is used for orthopedic and dental purpose, the bone bonding ability is mainly evaluated by studying its ability to form apatite on its surface in a body stimulated fluid (SBF) in which the ion concentrations are kept nearly equal to that of the human blood plasma. Apatite forming ability of any substance is used to predict the *in vivo* bioactivity of a material. This is done because any artificial material when implanted into the body gets encapsulated by a fibrous tissue isolating it from the surrounding bone. The essential requirement of a material to bond to a living bone is the formation of apatite on the surface which is bone-like in the living body that can be imitated in the SBF. These materials are believed to bond to living bone through a calcium phosphate layer. This is true till the material lacks any toxic or antibody reactions inducing component. There are cases when few materials bond to living bone without any detectable apatite in surfaces. SBF is useful in prediction of *in vivo* bioactivity not only qualitatively but also quantitatively [12].

CHAPTER 2

LITERATURE REVIEW

This chapter is about the importance of role played by porous Ti- medical implants in clinical applications. The Ti implants must be biocompatible, biodegradable and osteoinductive. Such implants can be fabricated using certain fabrication techniques. Titanium and its alloys have impressive mechanical properties and biocompatibility. On implantation, implants exhibit direct contact with bone. But smooth titanium implants show weak bonding to bones even in unloaded conditions [13]. To combine mechanical properties with bone-bonding abilities, titanium metal has been coated with certain bioactive materials. Hydroxyapatite (HA) plasma spray coating is a preferred method for orthopaedic implants [14]. Histological examinations have revealed that HA coated implants in direct contact with bone for 3-4 weeks after implantation produce areas of direct contact between implant and host-bone in addition to intervening fibrous tissue. Alkali and heat treated implants show better osteoconductive nature in accordance with HA coated implants than those by untreated implants. There is submicron level trabecular and irregular structures on the implant surface which can be put to use in orthopedic implants [15].

Surface properties of titanium have been altered with calcium phosphate or oxide-coating, alkali treatment and ion-implantation. Plasma spraying and amino group (NH_2^+) ion implantation provide bio-compatibility [16,17]. Cell behavior have been greatly been tested with surface modified titanium by observing morphological behavior, cell proliferation as well as differentiation. Biocompatibility of a biomaterial is closely related to cell interaction and adhesion onto the surface. Plasma spraying results in an increase in the surface roughness and macro-pores of plasma-sprayed porous Ti (PST) and plasma-sprayed and ion-implanted Ti (PSIT). Amino-group ion implantation leads to formation of thicker surface oxide layer with a little amount of nitrides of sand-blasted and ion-implanted (SIT) and PSIT. Cells attach, spread and proliferate on bottom of culture plates and display polygonal spindle shaped morphology on

the surface- modified Ti. After 5 days, cells show colonized patches by spreading, cell multilayers that grow into macro-pores (Porous Ti) and extracellular matrix (PSIT) which improves after 7 days. Surface modified Ti is non-toxic as well as a prerequisite for cell proliferation and osteogenesis [18].

Porous bioactive titanium by chemical and thermal treatments induces bone formation even at non-osseous sites in the absence of any osteoinductive agent. Chemically treated implants have better *in vitro* apatite forming ability and *in vivo* osteoconductive ability. Interconnectivity of pores allow cell and tissue invasion. The osteoconductive ability of bioactive titanium is found to be same as hydroxyapatite and bioactive glass [19]. The optimal pore diameter for *in vivo* osteoconduction is in the range 150-500 μm [20]. A combination of porous Titanium with growth factors might be quite useful for the use under load-bearing conditions in tissue engineering.

Information about grit-blasted, blasted and etched, and dual-etched c.p. Titanium implants show that bone formation is facilitated at these topographically modified surfaces is better than the machined c.p. titanium implants [21]. Surface group C-F displays better proliferation than those without any treatment. Increasing Fluoride treatment has been found to show decrease in cell proliferation. Direct bone-to-bone implant contact at large grit-based implants are 34.21% ± 12.08 and with fluoride treatment is 55.45% ± 22.01. Human osteoblastic cells grown on fluoride composites show better proliferation and differentiation [22]. Ionic modification alters the adsorption of the adhesive proteins. Direct effect of fluoride ions with osteoblastic cells or indirect fluoride ion effects on adsorption of protein and cell adhesion at c.p. titanium might be

precipitation of calcium phosphate that influence local calcium concentrations and thus cell behavior [23].

The main disadvantage with conventional methods of fabricating porous titanium including solid-state foaming, loose pack sintering, etc lies with the need of complex manipulation or failure in achieving the ideal porous framework [5]. Pore size of a biomaterial plays an important role in bone formation both *in vivo* and *in* vitro. Large pore lead to direct osteogenesis, whereas small pores lead to a osteo-chondral formation before any osteogenesis[24]. Using 10, 25, 40% of H_2O_2 as foaming agents, porous titanium are fabricated resulting in 48, 64 and 76% porosities [4] When porosity of porous titanium is 64%, the compressive strength is found to be 102 ± 10 MPa being comparable to our natural cortical bone [25] implicating the material to be applicable for load-bearing purposes. With compressive strength value above 20 MPa, having high interconnected porous structure, those titanium implants with porosity 76% find their use as orthopaedic implants and tissue engineering scaffolds [26].

CHAPTER 3

MATERIALS AND METHODS

3.1 Titanium specimen preparation

The Titanium sample obtained as sponge from the intermediate stage of Kroll's process (supplied by MIDHANI, India) is washed with acetone followed by water for 10 minutes each in an ultrasonic cleaner and then chipped into smaller pieces repeating the process of ultrasonication.

3.2 Surface treatments

3.2.1 Alkaline treatment

Porous bioactive titanium was prepared by chemical treatment of immersion of specimen in a 5M 20ml aqueous NaOH solution at 60^{o}C for 24h followed by immersion in distilled water for further 48h at 40^{o}C. Specimen was removed, allowed to dry and then stored carefully.

3.2.2 Dual Acid Treatment

Dual acid treatment was performed by soaking an untreated sample in an aqueous solution and H_2SO_4 and HCl in the ratio of HCl: H_2SO_4: H_2O as 3:3:9 for 20 minutes. The sample is washed with distilled water and dried at room temperature for 24h.

3.2.3 Citric Acid Treatment

Titanium surface was made bioactive by immersing it in 0.2M citric acid for 5h at room temperature, washing in distilled water and then stored carefully after drying.

3.2.4 Fluoride Treatment

Untreated samples were exposed to calcium fluoride (0.232g) etched in sulfuric acid (0.392g or 0.306ml), 40ml 0.1M solution at 120^{o}C for 1h. The sample is removed carefully and then stored. The governing equation for this reaction is:

$$CaF_2\,(s) + H_2SO_4\,(l) \rightarrow CaSO_4\,(s) + 2HF\,(g) \uparrow$$

3.3 XRD Analysis

After the surface treatments of the samples, the surface structural changes were examined by an X-ray diffractometer (PAN ANALYTICAL PW 3040 X'Pert MBD).

3.4 SEM Analysis

The surfaces of the treated as well as untreated samples were examined by scanning electron microscopy (SEM, NOVA NANO SEM_450 field emission microscopy). This examines the surface structural changes and morphology of the surface treated titanium samples.

3.5 Porosity Measurement

3.5.1 Surface Porosity Measurement

The untreated sample was polished in 1/0, 2/0, 3/0, 4/0 emery sheet for removal of passive oxide layer and etched with Kroll's Reagent for 30s. The sample is then removed from the solution and dried before mounting on the microscope. The optical microscope (METISCOPE-I, Chennai Metco Pvt Ltd) was used for capturing images at 100X, 200X and 400X magnifications. Envision 5.0 image analysis software was used to measure the surface porosity.

3.5.2 Bulk Porosity Measurement

Studies have revealed that porosity is an important parameter for the evaluation of an implant to be osteoconductive. Experiment to test the bulk porosity of the untreated titanium sample was carried out using the Archimedes principle which is expressed as,

$$\Phi = (V - V_s)/ V = V_p/V$$

where, V = bulk volume

V_s = volume of solid

V_p = pore volume

3.6 Assessment of In vitro Bioactivity (immersion in SBF)

Bioactivity of the untreated and treated samples was evaluated by examining the apatite formation on the surfaces in SBF ((Table I). SBF was prepared by dissolving NaCl, $NaHCO_3$, KCl, $K_2HPO_4.3H_2O$, $MgCl_2.6H_2O$, $CaCl_2$, Na_2SO_4 into distilled water buffered at 7.40 with 1M-HCl and *Tris* at 36^oC. Surface treated samples were immersed in SBF for 4 weeks. After soaking, the samples are dried in a clean bench. The morphology of the surface was observed under a scanning electron microscope (JEOL-JSM-6480 LV) at 15kV. This surface characterization confirms apatite formation.

Table I

Ion Concentrations of Simulated Body Fluid (SBF) and Human Blood Plasma

	Concentration (mM)							
	Na^+	**K^+**	**Mg^{2+}**	**Ca^{2+}**	**Cl^-**	**HCO^{3-}**	**HPO_4^{2-}**	**SO_4^{2-}**
SBF	142.0	5.0	1.5	2.5	148.8	4.2	1.0	0.5
Blood Plasma	142.0	5.0	1.5	2.5	103.0	27.0	1.0	0.5

3.7 Protein Adsorption Study

Amount of protein adsorbed on a sample is a vital factor in influencing cellular interactions *in vitro* as well as *in vivo.* Samples were treated with 1ml of BSA protein standard (1mg/ml protein in phosphate buffer solution, PBS) followed by keeping in an incubator at 37^oC for 24h. The samples are then removed and washed three times using PBS for removing the non-adsorbed

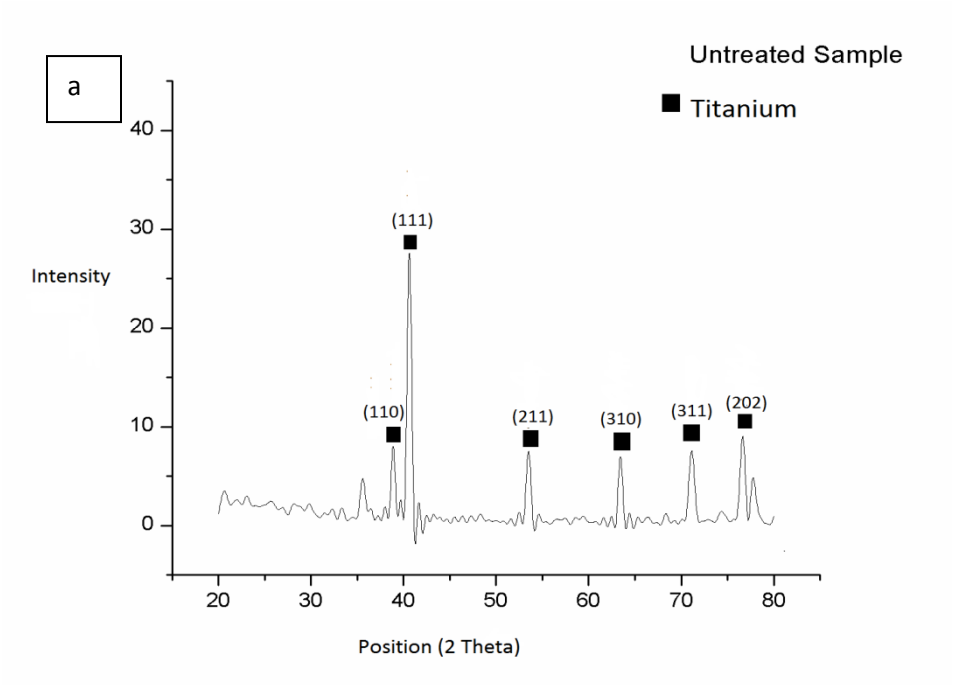
protein. Bradford assay was used to quantify the adsorbed protein amount. 50µl of non-adsorbed protein was mixed with 500µl of Bradford reagent. The protein concentration was determined by UV spectrophotometer at 595nm using a previously obtained standard curve. This helped in the accurate determination of concentration of adsorbed protein on different samples.

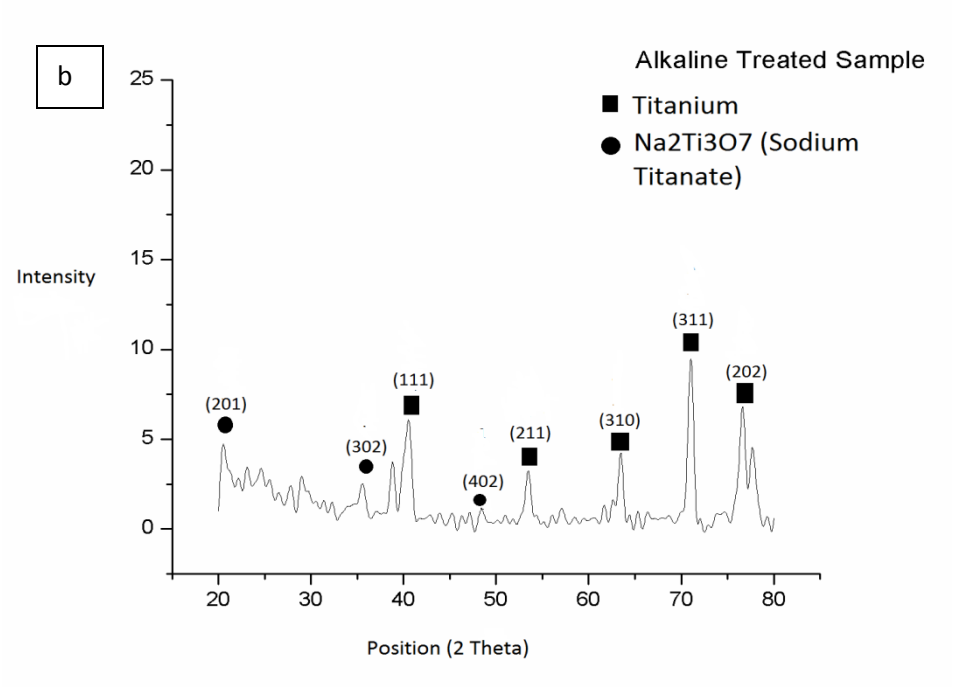
CHAPTER 4

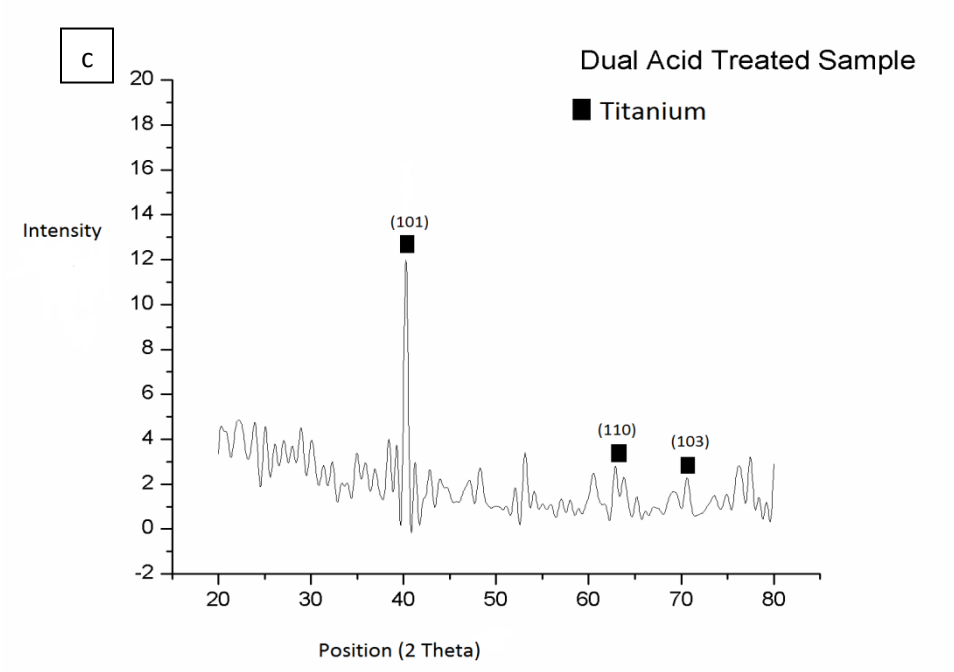
RESULTS AND DISCUSSION

4.1 XRD Analysis

The XRD patterns of various surface treated Ti-samples are shown in Fig 1 along with the pattern of the untreated sample. The diffraction peaks correspond to Titanium without showing any new phase as a result of chemical treatment. In the alkali treated sample, there is a decrease in the intensity which might be due to the sodium titanate (gel) on the surface detected by XRD. The fluoride treated sample shows peaks corresponding to Titanium as well as calcium sulfate.




b
Alkaline Treated Sample
Titanium
Na2Ti3O7 (Sodium Titanate)
Intensity
Position (2 Theta)
(201)
(302)
(111)
(402)
(211)
(310)
(311)
(202)


c
Dual Acid Treated Sample
Titanium
Intensity
Position (2 Theta)
(101)
(110)
(103)

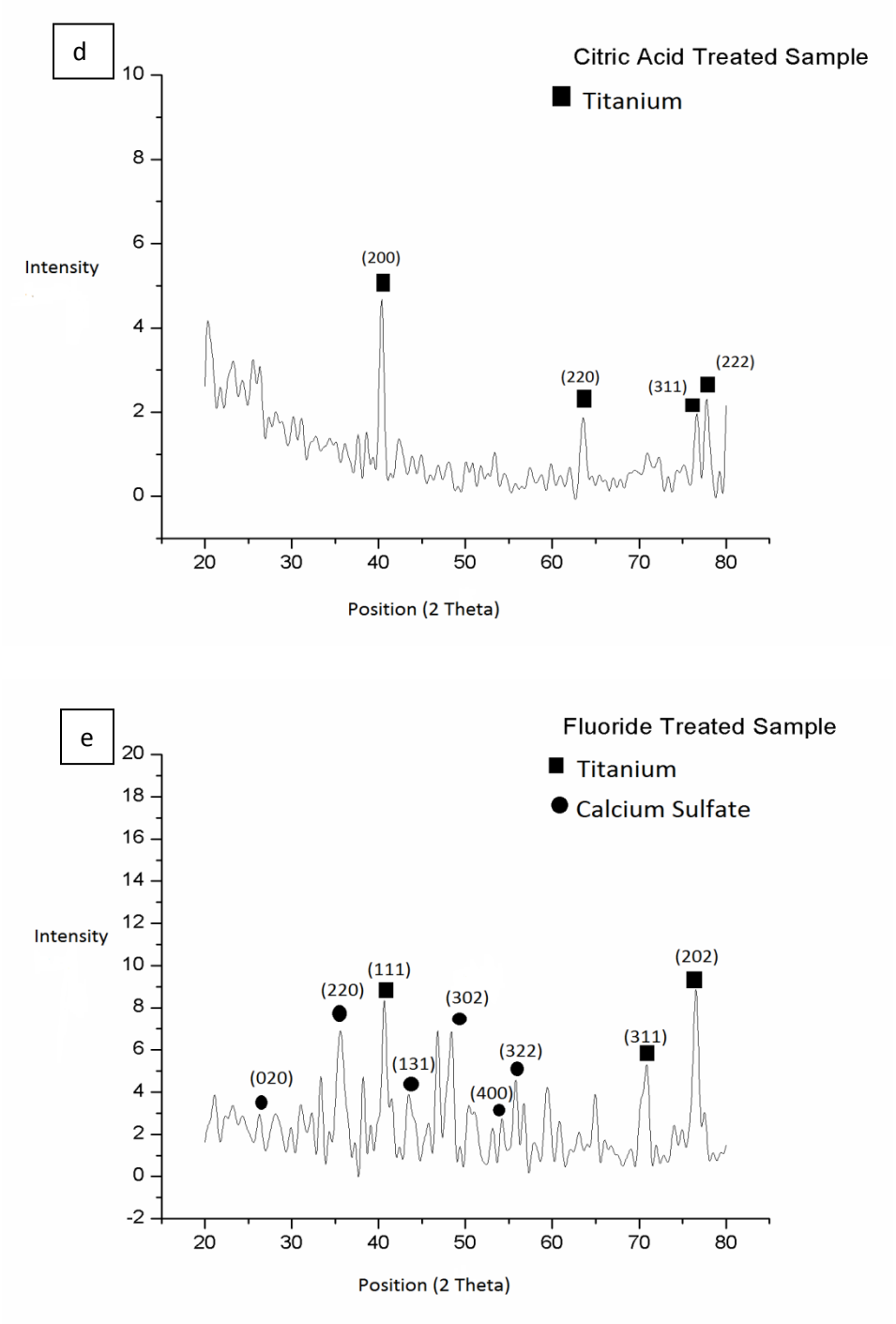
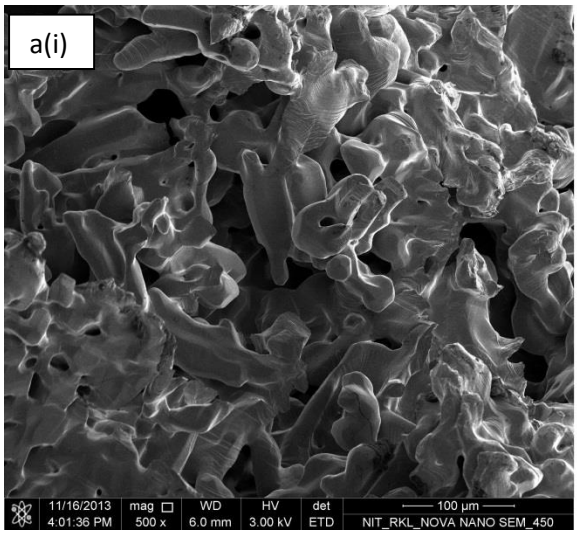


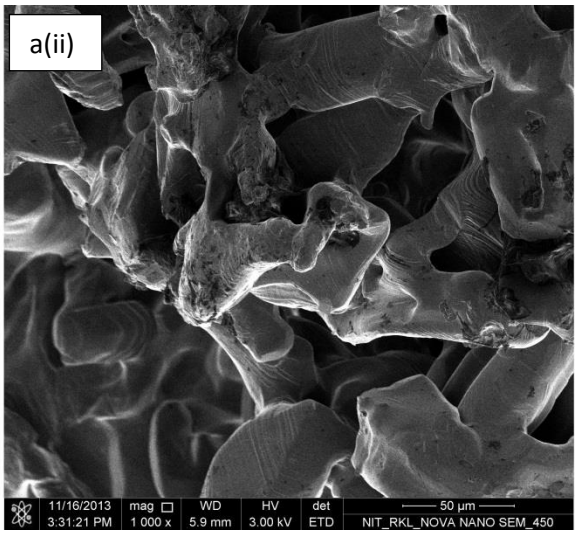
Fig 1: XRD pattern of various surface treated Ti-Samples. (a) Untreated Sample (b) NaOH Treated Sample (c) Dual Acid Treated Sample (d) Citric Acid Treated Sample (e) Fluoride Treated Sample

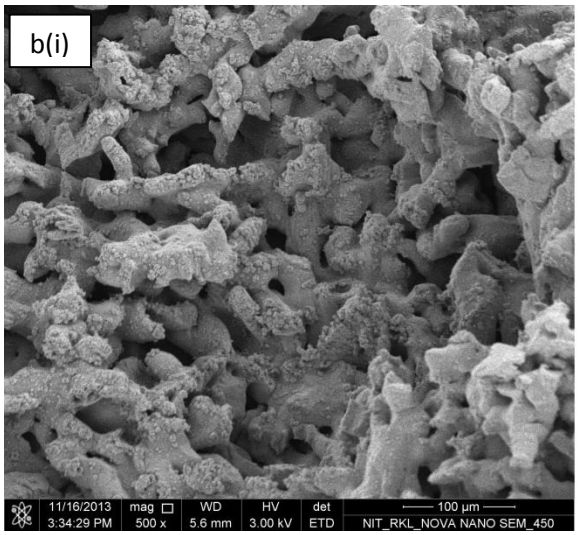
4.2 SEM Analysis

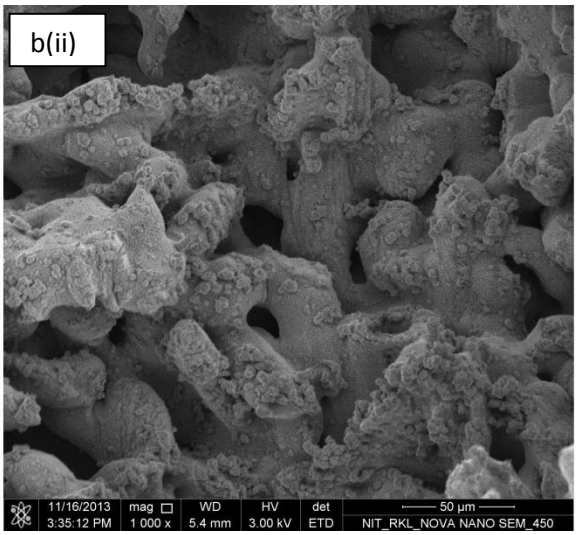
Fig. 2 shows the SEM images of the different treated Ti samples in comparison with the untreated one. The SEM images show the differences in the surface morphologies of the samples. The untreated sample (Fig 2(a(i))) was revealed to have a smoother surface. Higher magnification (Fig 2(a(ii))) showed the smooth pore-wall of sample with 3D interconnected pores. Alkali treated sample (Fig 2(b)) had few sodium titanate on the surface. Titanium forms a passive oxide layer on contact with air. This film is non-uniform and is composed of TiO_2 outer layer and an intermediate TiOx before the pure Ti layer. Alkali treatment of the sample doesn't remove the entire passive layer though. The images also project the presence of interconnected pores that is helpful in osteogenesis during bioactivity studies. Microporous structures are found on the pore-wall surfaces of this chemically treated implant. As the entire sample was immersed in NaOH, this effect extends throughout the irregular inner pores of the sample. The dual acid treated sample (Fig 2(c(i))) was found to have rough surface orientations. Further higher magnifications (Fig 2(c(ii))) show the removal of entire passive oxide layer causing micro-roughness increasing the surface area of the sample. Fig 2(d) shows the surface morphology of the citric acid treated sample where not much difference was found due to titanium being almost inert to citric acid although minor rough orientations are found which owe to removal of passive oxide layer. SEM image Fig 2(e(i)) reveals white bulky needle-like substance with complete structure and stable dimensions. This is due to the reaction that resulted in anhydrous calcium sulfate crystals visible as white flaky substance to the naked eye on the sample surface. With further magnification in Fig 2(e(ii)) it is noticed that the length to diameter ratio is quite high

which might add impressive mechanical properties to Ti. These calcium sulfate whiskers enhance the interconnected 3D porosity for better cell proliferation, adhesion and nutrient supply.









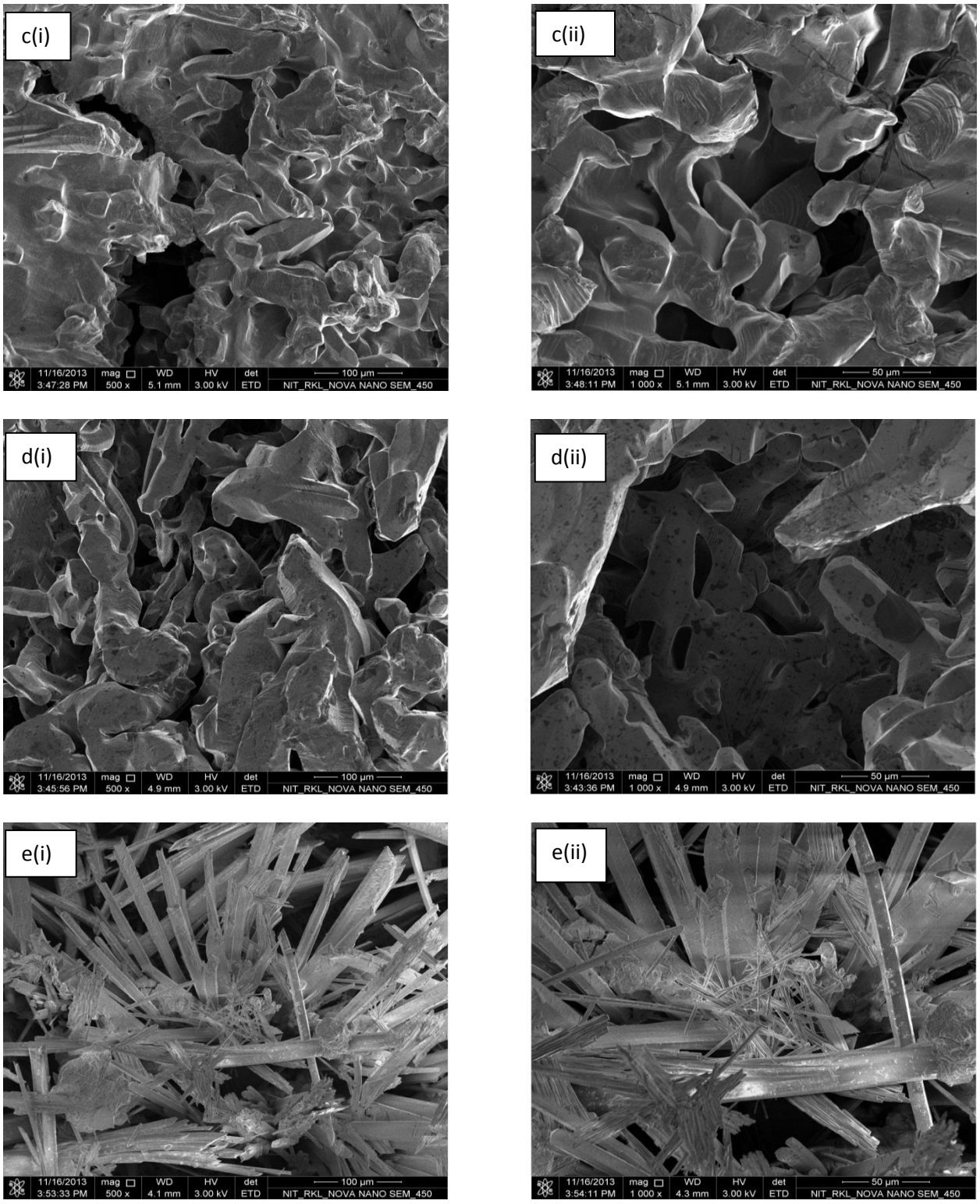
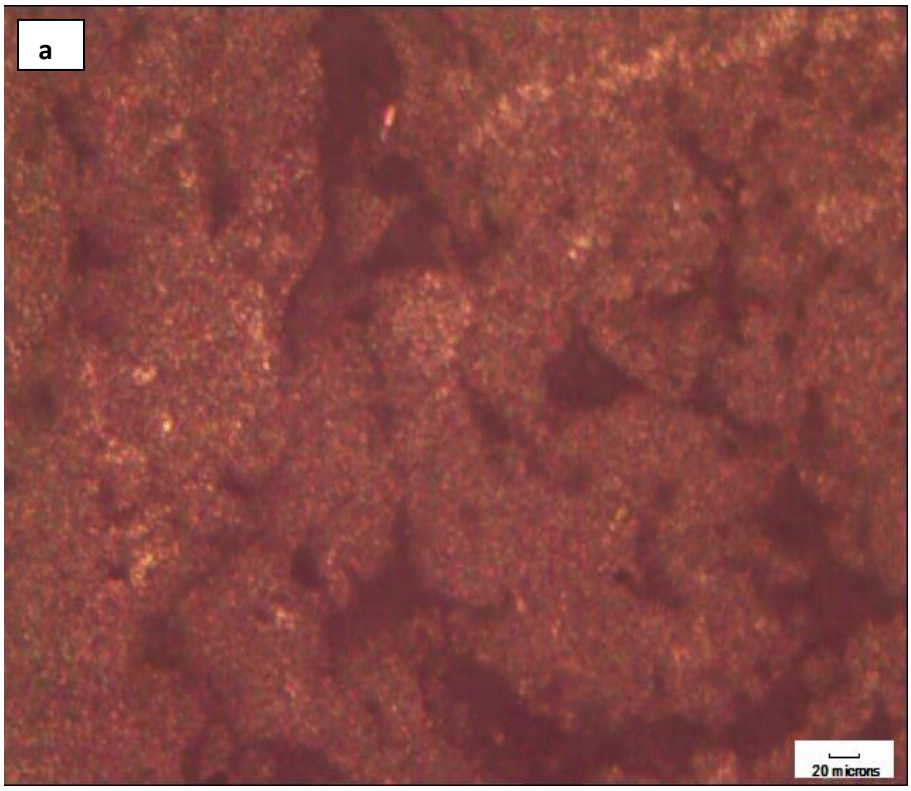


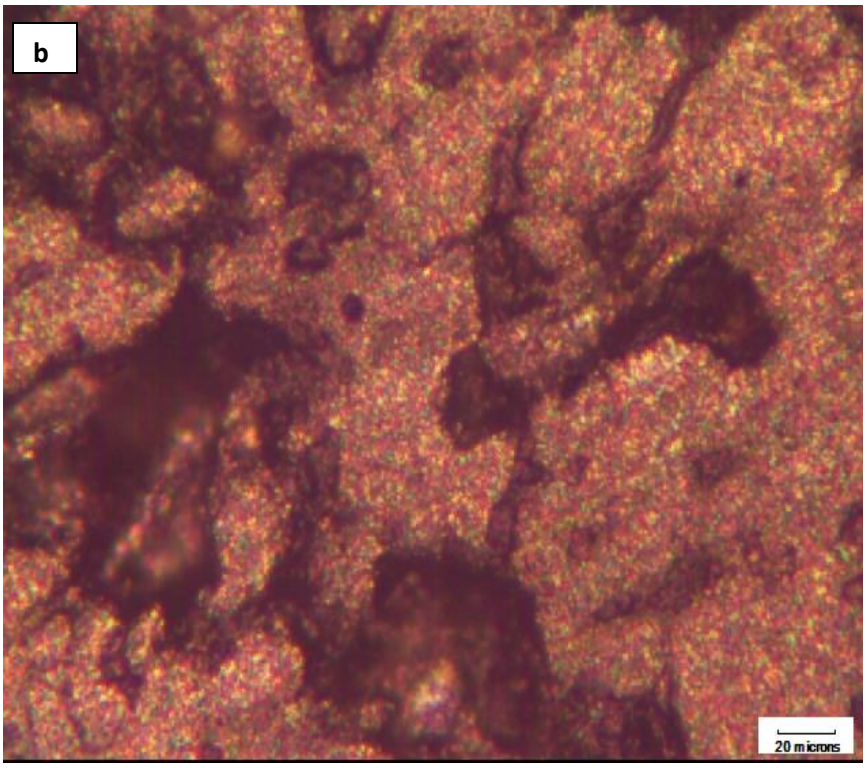
Fig 2. Scanning electron microscope (SEM) micrographs of surface treated titanium at 500X and 1000X. (a) Untreated Ti-sample. (b) Alkali Treated Ti-sample. (c) Dual Acid Treated Ti-sample. (d) Citric Acid Treated Ti-sample. (e) Fluoride Treated Ti-sample.

4.3 Porosity Measurement

4.3.1. Surface Porosity Measurement

The captured pictures are analyzed using ENVISION-5.0 software for the surface porosity. The surface porosity was found out to be around 41.36% as shown in Fig 4. There might be a loss in the surface porosity due to polishing.





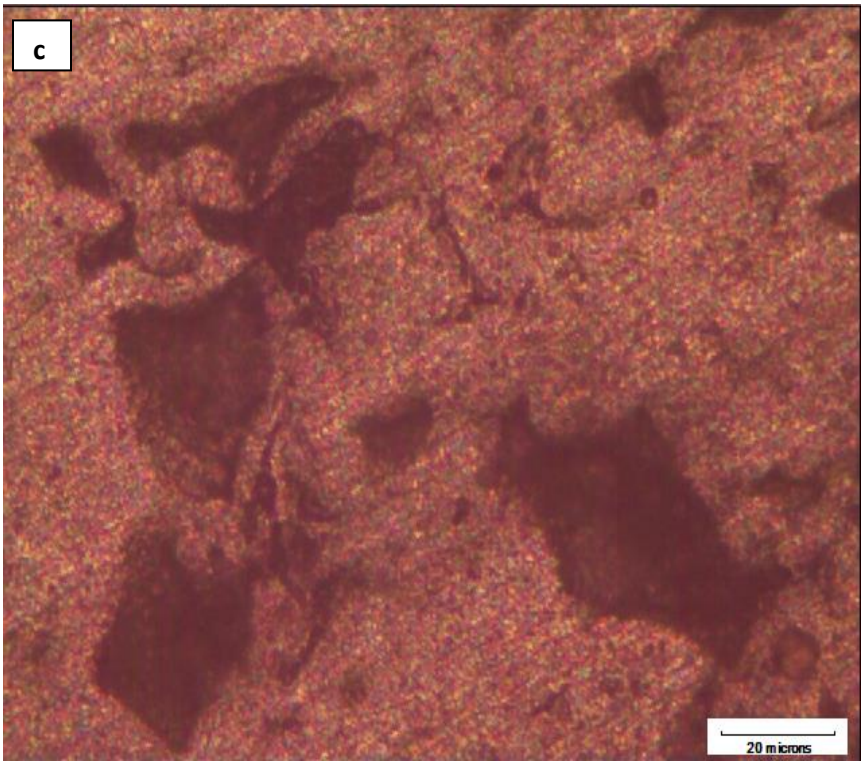


Fig 3: Surface porosity measurement of untreated titanium using optical microscope at (a) 100X (b) 200X (c) 400X

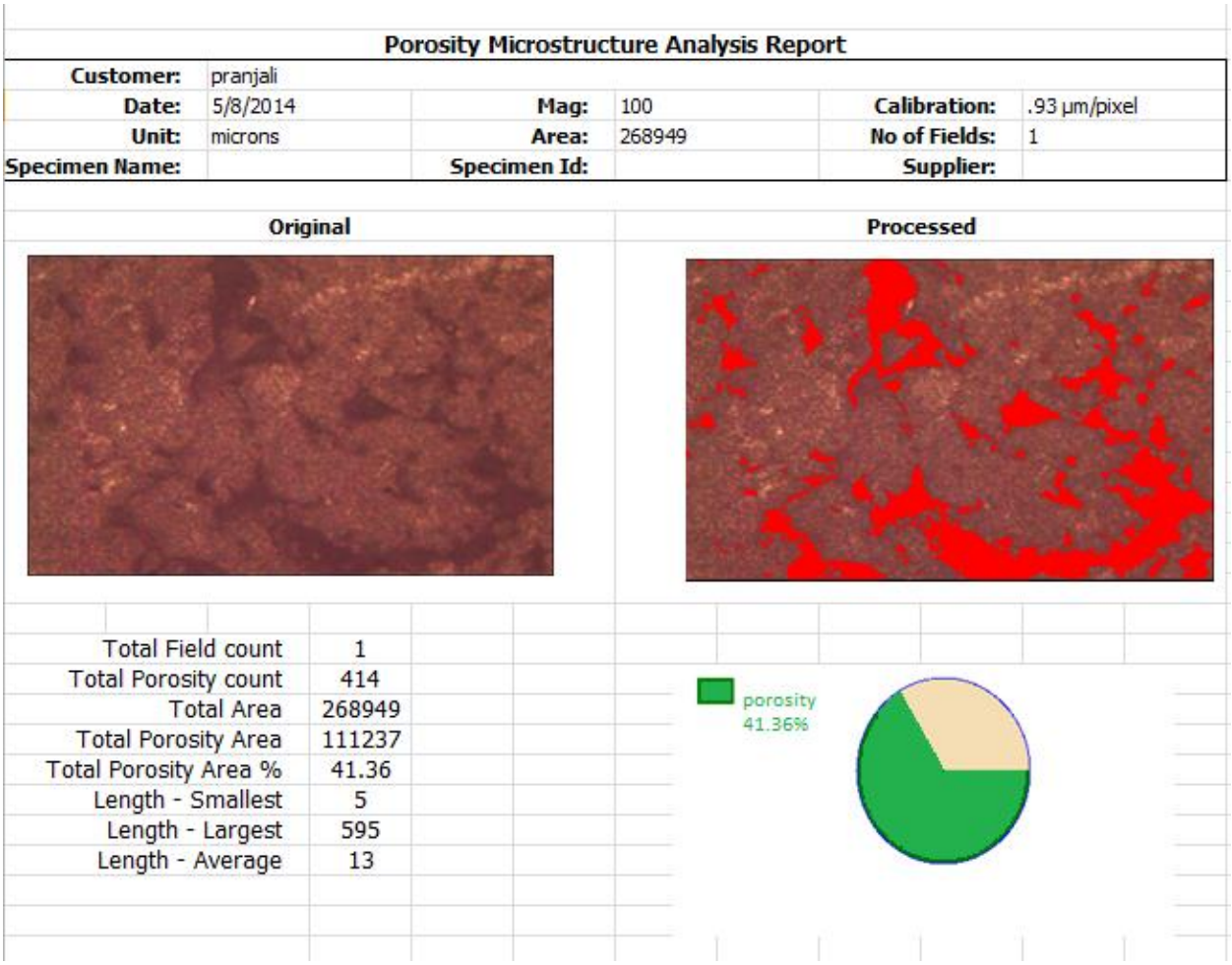


Fig 4: Surface porosity analysis of untreated titanium

4.3.2 Bulk Porosity Measurement

Porosity in the morphology has been found to enhance cell attachment, proliferation and expression of various matrix components. These pores in micro-scale increase the surface area

for protein adsorption. The porosity analyzed by Archimedes principle gave the porosity of the untreated sample to be averaged at 50.347% according to the data in Table II.

TABLE II

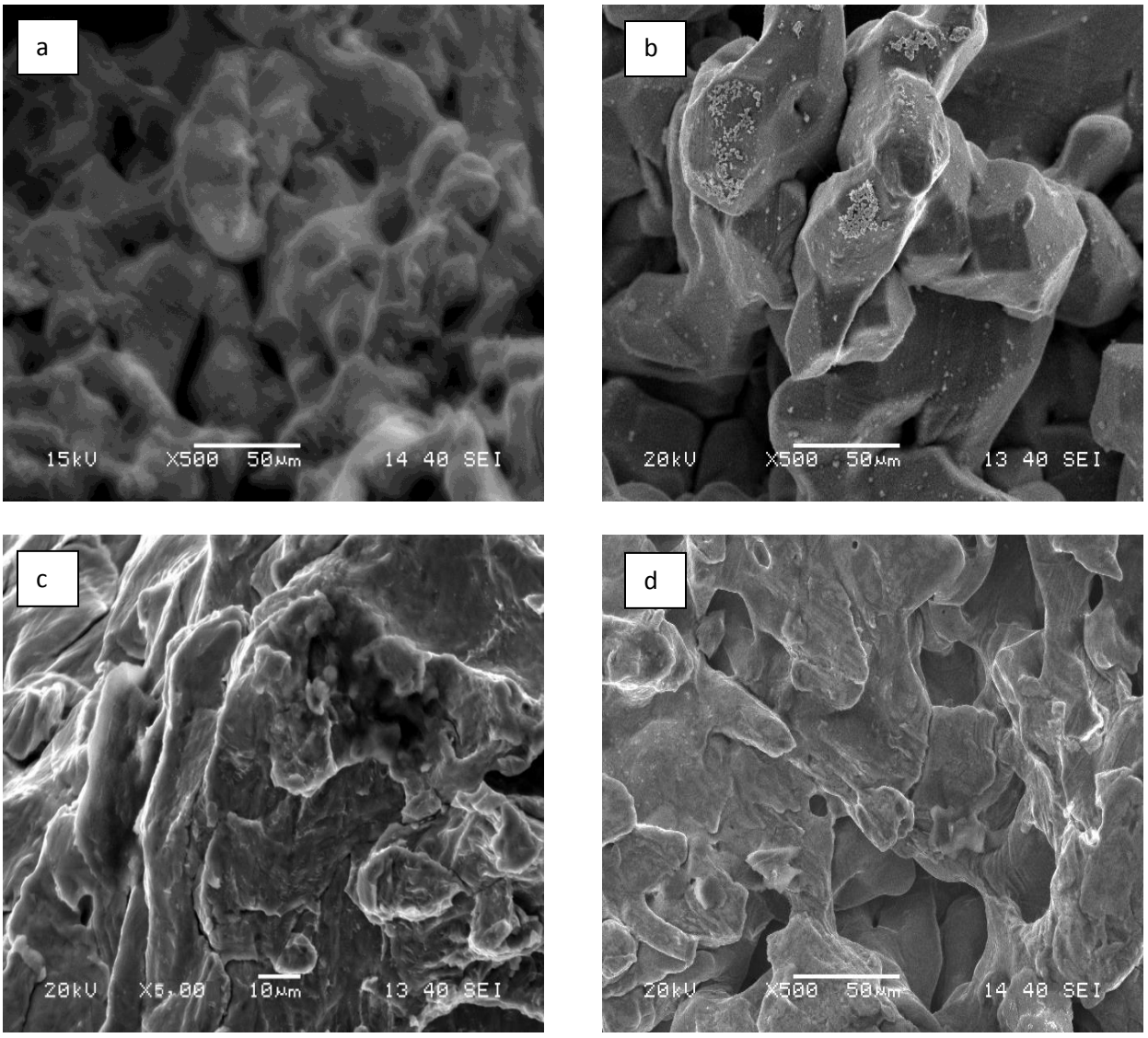
Porosity measurement of the untreated sample

Sl No.	**Mass(g)**	**Density(g/cc)**	**Volume(cc)= Mass/Density**	**Volume Dispersed= V(cc)**	**Porosity=(V-V_s)/V= V_p/V**
1.	2.679	4.506	0.5945	1.2	0.5046
2.	2.710	4.506	0.601	1.21	0.5033
3.	2.692	4.506	0.597	1.2	0.5025

4.4 In vitro bioactivity

Fig. 5 shows the SEM examination of the treated Ti-sample along with the untreated one after being immersed in SBF for 4 weeks. The deposit of spherical particles on the untreated sample (Fig 5 (a)) was observed as few hydroxyapatites (HA) patches on the surface. This is due to the smooth surface of the sample leading to undesirable HA formation. The alkali treated sample (Fig 5 (b)) showed an increase in the HA patches due to an increase in the surface area. Na^+ released forming sodium titanate act as nucleating sites for apatite. The dual acid treated sample (Fig 5 (c)) produce valleys on the surface promoting roughness that turn to be the preferred sites for growth of HA. For the citric acid treated sample (Fig 5(d)) not much apatite formation was found on the surface and only few scattered HA patches were visible. Deposit was found to be

the maximum with globular apatite on the surface with the fluoride treated sample (Fig 5 (e)). The calcium sulfate increased the surface energy that facilitates nucleation and apatite (dense) formation.



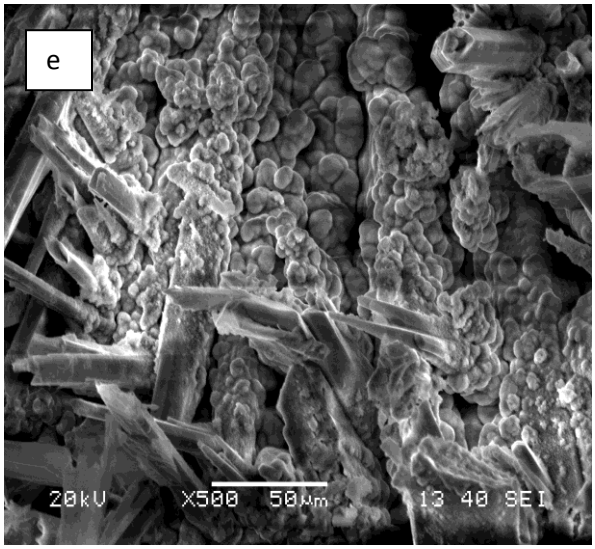


Fig 5: SEM micrographs of surface treated titanium samples in SBF for 4 weeks. (a) Untreated sample (b) Alkaline Treated sample (c) Dual Acid Treated Sample (d) Citric Acid Treated Sample (e) Fluoride Treated Sample

4.5. Protein Adsorption

BSA adsorption is favorable for cell attachment and proliferation which is prominent from the protein adsorption on surface treated Ti-samples. Enhanced cell attachment in Ti-samples is associated with greater binding affinity of cells for BSA. Table III gives the data about protein adsorption by various samples.

TABLE III

Protein Adsorption in Various Treated Samples

SL. NO.	SAMPLE NAME	ADSORBED PROTEIN (µg/ml)
1.	Untreated Sample	1720.28
2.	NaOH Treated Sample	1866.74
3.	Dual Acid Treated Sample	1643.06
4.	Citric Acid Treated Sample	1420.22
5.	Fluoride Treated Sample	1616.44

The maximum amount of adsorbed protein was found with the alkali treated Ti-sample. A correlation between surface wettability and protein binding is a result of the presence of water molecules. Amount of adsorbed proteins is significantly higher on hydrophilic surface than a hydrophobic surface. During alkali treatment the TiO_2 passive layer dissolves into the alkali solution due to hydroxyl groups that are corrosive. This occurs simultaneously along with Ti hydration. Further hydroxyl attack leads to hydrates that are negatively charged on the Ti-substrate. These combine with alkali ions in the solution to form an alkali titanate hydrogel layer. This leads to surface hydration of Ti by alkali treatment [27, 28]

CONCLUSION

A very essential condition for an implant to bond to living tissue is the formation of a biologically active bonelike apatite on the surface. This apatite can be reproduced on material surface *in vitro* in SBF. Porous Ti and surface treated porous Ti show to form the bonelike apatite layer on their surfaces even in living body, and bond to human bone through this layer. There is surface change of Ti-sample when they are chemically treated with various methods. Surface change is also induced onto the surface when apatite formation takes place on the surface in SBF. During alkali, dual acid and citric acid treatment the passive oxide layer partially gets removed into the respective solutions because of the corrosive attack of hydroxyl group. The apatite formation in surface modified titanium indicates that the samples are inductive for bioactivity. Each reaction parameter affects resultant induction ability of apatite formation for each Ti-sample. Optimization of these factors will help to develop functionally gradient bioactive Ti for useful clinical applications. Maximum apatite deposit was found on fluoride treated Ti-sample due to the calcium sulfate whiskers which gives more surface roughness; an indication of high surface energy. XRD analysis showing diffraction patterns for titanium indicated there is no phase change in the base sample due to any surface treatment. Protein adsorption is a vital requirement for cell adhesion and proliferation. Ti- sample with alkali treatment has a hydrolyzed surface which enhanced the protein adsorption. Few of these treated surface modified samples are commercially available for clinical use. But the exact use of the surface chemistry and topography comes into play during the osseointegration of the Ti-implants. The future of this implantology is aimed at standardizing the surface chemistry and topography. The ultimate aim is the enhancement of osseointegration of the implants for the loading, impressive properties and long-term fixation purpose.

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